

Water Potential of Aqueous Polyethylene Glycol

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ABSTRACT

Water potential (ψ_w) values were determined for aqueous colloids of four molecular sizes of polyethylene glycol (PEG) using freezing-point depression and vapor-pressure deficit methods. A significant third-order interaction exists between the method used to determine ψ_w , PEG molecular size, and concentration. At low PEG concentrations, freezing-point depression measurements result in higher (less negative) values for ψ_w than do vapor-pressure deficit measurements. The reverse is true at high concentrations. PEG in water does not behave according to van't Hoff's law. ψ_w is related to molality for a given PEG but not linearly. Moreover, ψ_w varies with the molecular size of the PEG. It is suggested that the ψ_w of PEG in water may be controlled primarily by the matrix forces of ethylene oxide subunits of the PEG polymer. The term matricum is proposed for PEG in soil-plant-water relation studies.

PEG [an inert, nonionic, long-chain polymer: $\text{HOCH}_2\text{—}(\text{CH}_2\text{—O—CH}_2)_x\text{—CH}_2\text{OH}$ (3)] has been widely used to maintain experimental media at predetermined ψ_w values (2, 7, 11, 14-16). Several researchers have reported that PEG has toxic effects on plants (5, 10). Lagerwerff *et al.* (8) attributed PEG 6,000 toxicity to associated heavy metals and recommended dialysis or passage through ion exchange columns to remove these impurities. Lawlor (9) suggested that PEG 1,000, 4,000, and 20,000 caused plant desiccation by blocking pathways of H_2O movement.

In addition to possible toxic effects, debate exists concerning the appropriate method to use for determining ψ_w of PEG media (8). Applegate (1) noted that the freezing-point depression of PEG 4000—Hoagland solutions of different concentrations deviated from the expected value predicted by van't Hoff's law. Lagerwerff *et al.* (8) reported that ψ_s of PEG—Hoagland solutions obtained using freezing-point depression methods were 20 to 40% lower (more negative) than those obtained using a thermocouple psychrometer. Janes (6), using freezing-point depression methods, reported that he obtained reliable ψ_s for PEG 400, and that his results closely agreed with those obtained by others using a thermocouple psychrometer (12).

A review of the literature on the use of PEG for stimulating ψ_w s and plant water stress suggests that, although PEG is widely used, the relationships between PEG concentration, mol wt, and method of determining ψ_w are not well-understood. The objectives of this research were: (a) to identify differences which may occur in PEG ψ_w measurements obtained using freezing-point depression

and vapor-pressure deficit methods and (b) to clarify the components of ψ_w active in PEG media.

MATERIALS AND METHODS

A laboratory advanced osmometer, model 3L (Advanced Instruments Inc., Needham Heights, MA) was used to determine the freezing-point depression of media. Vapor-pressure deficit measurements were obtained for the same media with a dew-point microvoltmeter, model HR-33, and thermocouple chamber, model C-51 (Wescor Inc., Logan, UT). All dew-point measurements were done at 23°C. Both instruments were calibrated using the same standards. Readings in mosmol and μV were converted to corresponding ψ_w values for statistical analysis. Polyethylene glycol was purchased from J. T. Baker Chemical, Phillipsburg NJ.

Test media were made by adding 50, 100, 150, 200, 250, 300, 350, and 400 g PEG of four molecular sizes to 1000 g distilled H_2O . PEGs used were PEG 1,000 (mol wt, 950-1,050), PEG 4,000 (mol wt, 3,000-3,700), PEG 6,000 (mol wt, 6,000-7,500), and PEG 20,000 (mol. wt, 15,000-20,000). All measurements were replicated five times.

Curvilinear regression models were determined for each method and PEG. An analysis of variance using a $2 \times 3 \times 8$ factorial model with five replicates was used to determine differences between the two methods, three PEGs (1,000, 4,000, 6,000), and eight concentrations. Since PEG 20,000 would not freeze at 350 and 400 g/1,000 g H_2O concentrations, differences in measured ψ_w between the two methods were analyzed separately. Differences were considered significant at the 95% confidence level.

RESULTS AND DISCUSSION

The relationship between ψ_w values obtained for PEG media and the method used for determination appears much more complicated than previously assumed (Figs. 1 and 2). The analysis of variance indicates a significant third-order interaction between the method used and the molecular size and concentration of PEG (Table I). The freezing-point depression method produced ψ_w estimates from 26 to 44% higher (less negative) than vapor-pressure deficit estimates at low concentration (50 to 150 g/1,000 g H_2O) and from 14 to 20% lower (more negative) estimates at high concentrations (300 to 400 g/1,000 g H_2O) for PEG 1,000, 4,000 and 6,000 (Fig. 1). ψ_w estimates based on freezing-point depression measurements for PEG 20,000 were higher than (less negative), or the same as, those obtained using vapor-pressure deficit measurements. The failure of freezing and psychrometric methods to agree at low concentrations (near pure H_2O) can be attributed to the difficulty in obtaining zero ψ_w readings. The vapor-pressure deficit curves do not extrapolate to zero ψ_w at zero concentration, whereas freezing-point depression curves tend to do so.

Van't Hoff's law predicts that the ψ_s of true solutions is directly a function of the number of particles in solution. Therefore, a

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² Abbreviations: ψ_w , water potential; ψ_s , solute potential; ψ_m , matric potential.

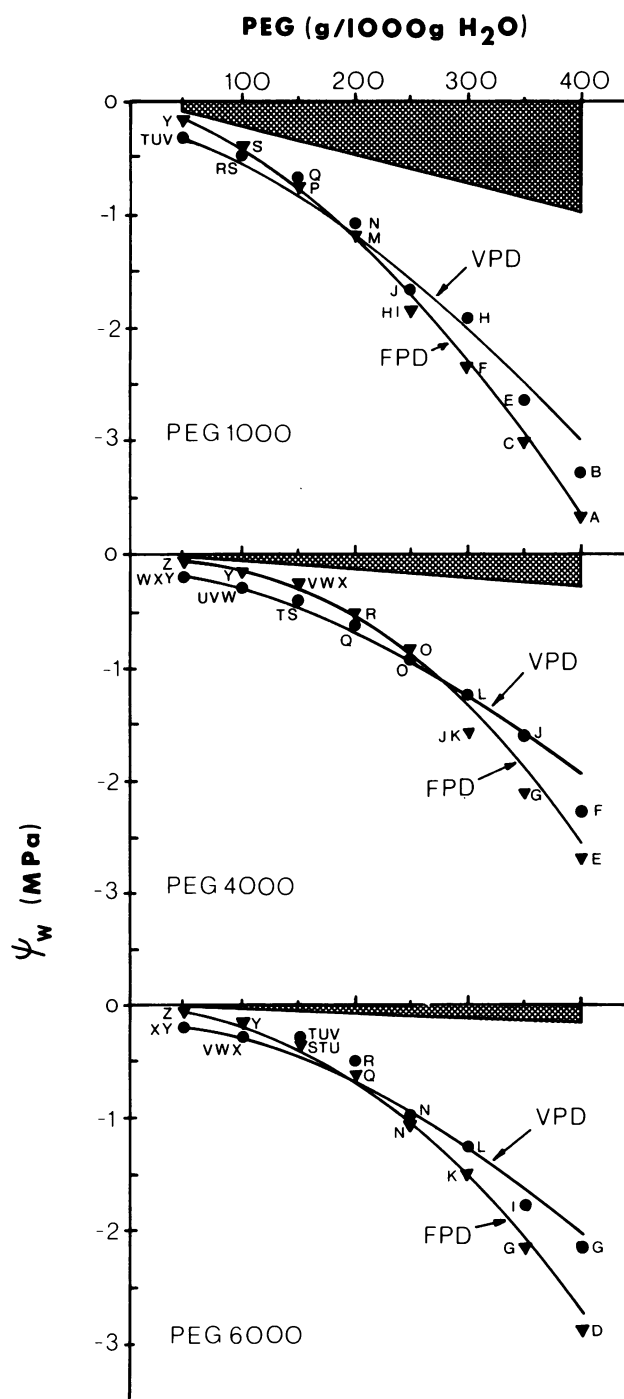


FIG. 1. Relationship between PEG concentration and ψ_w values determined by freezing-point depression (FPD) and vapor-pressure deficit (VPD) methods. Regression models of FPD and VPD data for PEG 1,000, 4,000, and 6,000, respectively, are: $Y = 0.0005X^{1.5}$ ($r^2 = 0.99$), $Y = 0.0025X^{1.17}$ ($r^2 = 0.95$); $Y = 0.00002X^{1.94}$ ($r^2 = 0.98$), $Y = 0.0014X^{1.19}$ ($r^2 = 0.93$); $Y = 0.00001X^{2.0}$ ($r^2 = 0.99$), $Y = 0.001X^{1.24}$ ($r^2 = 0.88$). Points followed by the same letter are not significantly different (Duncan's Multiple Range Test, $p > 0.05$). Shaded area is the expected ψ_s for a true solution.

mathematical model should provide nearly perfect prediction of this relationship. Freezing-point depression produced models with the highest coefficient of determination (Figs. 1 and 2). This indicates that the freezing-point depression method was less subject to machine and operator error. However, except for PEG

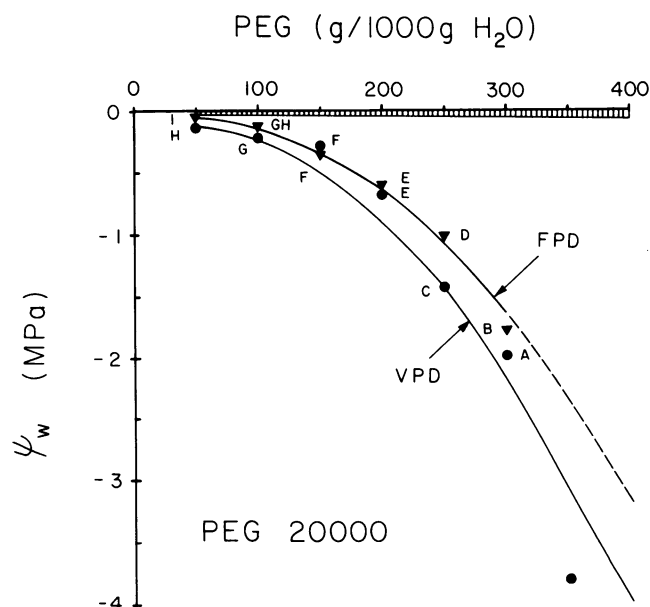
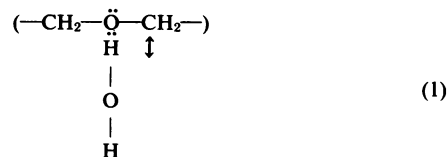


FIG. 2. Relationship between PEG concentration and ψ_w values determined by freezing-point depression (FPD) and vapor-pressure deficit (VPD) methods for PEG 20,000. Regression models are $Y = 0.00001X^{2.1}$ ($r^2 = 0.99$) and $Y = 0.00004X^{1.9}$ ($r^2 = 0.92$), respectively. Points followed by the same letter are not significantly different (Duncan's Multiple Range Test, $p > 0.05$). Shaded area is the expected ψ_s for a pure solution.

20,000, the vapor-pressure deficit method resulted in a more linear function of ψ_w and PEG concentration, as predicted by van't Hoff's law for true solutions. It should be noted that PEG of high mol wt in H_2O exhibits the Tyndall effect characteristic of colloids and, in that respect, is not a true solution.

The ψ_w , measured by vapor-pressure deficit, of each PEG medium was plotted against its molal concentration (Fig. 3). Molality and ψ_w are closely related for individual molecular sizes of PEG, but the ψ_w produced by a given molal concentration of PEG of different mol wt (having different polymer chain length) is by no means similar. For example, a ψ_w measurement of -2 megapascals was obtained for 0.02 molal concentrations of PEG 20,000, whereas a 0.3 molal concentration of PEG 1,000 is required for the same ψ_w . This is a 15-fold difference in concentration. It seems clear that PEG media do not observe van't Hoff's law. Indeed, the difference in molecular size between PEG 1,000 and PEG 20,000 is similar to the above ratio. This suggests that the total mass or total number of molecular subunits ($-CH_2-O-CH_2-$), rather than total number of particles (in this case, polymer chains), may be an important factor controlling ψ_w (Fig. 4).

In fact, it has been suggested (13) that, when ethylene oxide adducts are dissolved in H_2O , H_2O molecules are attracted by hydrogen bonding to the ether oxygen atoms as indicated below:



Moreover, systematic study of their analytical behavior has shown that some of the ethylene oxide adducts in aqueous solutions may be present as cation-active polyoxonium compounds:

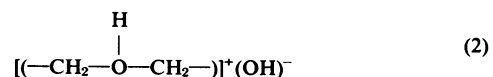
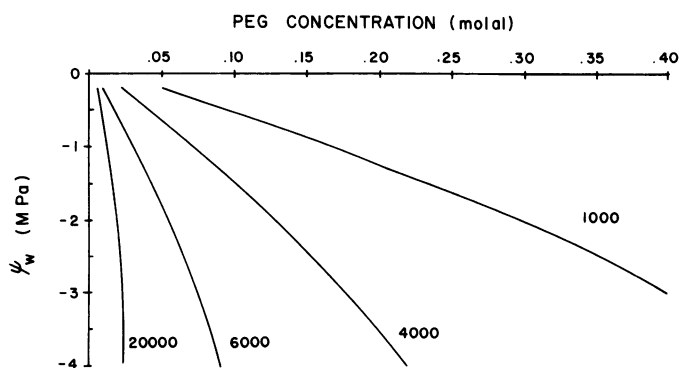


Table I. Analysis of Variance of ψ_w Measurements Obtained by Two Methods

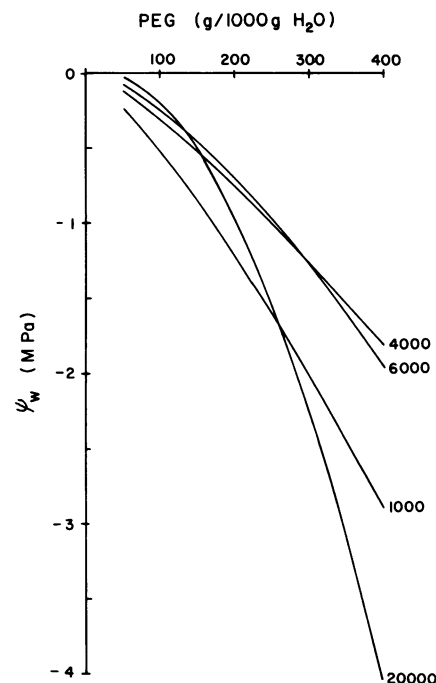
Source	Sum of Squares	D.F.	F value	Prob > F	C.V.
					%
PEG 1,000, 4,000, and 6,000					5.23
Method	1.22	1	312.8	0.0001	
PEG	18.15	2	2326.9	0.0001	
Method \times PEG	0.09	2	11.5	0.0001	
Concentration	198.15	7	7258.2	0.0001	
Method \times concentration	2.95	7	108.1	0.0001	
PEG \times concentration	4.80	14	87.9	0.0001	
Method \times PEG \times concentration	0.40	14	7.3	0.0001	
Error	0.76	192			
PEG 20,000					7.58
Method	0.20	1	64.5	0.0001	
Concentration	25.43	5	1640.6	0.0001	
Method \times concentration	0.28	5	18.1	0.0001	
Error	0.15	48			

FIG. 3. Relationship between molality of PEG of four mol wt and ψ_w measured by vapor-pressure deficit method.

An equilibrium between types 1 and 2 is believed to exist in aqueous solutions but the adducts react analytically according to type 2 (13). It seems that the positive charge is induced in the adducts only in the presence of compounds forming anions.

If the number of molecular subunits are determining ψ_w of PEG media, then matric forces may be involved. In fact, by using the molal concentration of PEG solutions and van't Hoff's formula, the osmotic contribution of PEG particles to total ψ_w can be calculated. These contributions are shown in Figures 1 and 2 by the shaded area under the ψ_s lines. At low concentrations of all PEGs, the contribution of ψ_s versus ψ_m to the ψ_w is more noticeable (Figs. 1 and 2). This suggests that, at very low concentrations, a PEG medium may be acting in a manner similar to that of a true solution. As the concentration of PEG increases, the relative share of ψ_s to the ψ_w diminishes rapidly. This dominant effect of ψ_m over ψ_s is more pronounced with higher mol wt PEG having longer but fewer chains (particles)/g.

In addition to the ψ_w components ordinarily considered by plant physiologists, there are other possibilities. It is certainly possible that a long-chain polymer may coil back on itself, and the change in entropy would depend on the flexibility of the chain (4). The configuration of a polymer chain is seldom exactly definable and, even if it were, a solvent molecule rarely would match it in size and shape. When a polymer such as PEG is immersed in an excess of H_2O , it apparently absorbs solvent until equilibrium is established between the thermodynamic interaction of solvent with polymer chain segments and the retractive forces arising from expansion of the network structure. It would also seem that as concentration increases, the chance of polymer-polymer interactions would become greater and further modify entropy, and, thus,

FIG. 4. Relationship between concentration by weight of PEG 1,000, 4,000, 6,000, and 20,000, and ψ_w measured by vapor-pressure deficit method.

the ψ_w .

Schöenfeldt (13) reported the formation of micellar structure for certain ethylene oxide adducts in an aqueous medium. Micellation resulted from hydrogen bonding between ethylene oxide subunits and H_2O molecules. He (13) further indicates that the degree of micellation is directly, although not linearly related to concentration. This information, along with the results of the study presented here (Figs. 3 and 4) indicate that, although osmotic forces are present, matric forces are the major component of ψ_w for PEG media.

Most investigators refer to PEG as an osmotic agent which alters the ψ_s of a solution. It has been recently suggested that the ψ_s of PEG media can be used to approximate soil ψ_m with respect to rates of seedling emergence (15). We feel that this terminology has led to a misunderstanding of the properties and usefulness of PEG in conducting research in plant- H_2O relations. In fact, PEG solutions of high mol wt and in concentrations used in physiological experiments behave like colloids, and matric forces are a

major component of the resulting ψ_w . Therefore, PEG should be referred to as a "matricum" rather than an osmoticum.

Freezing-point depression and vapor-pressure deficit methods apparently do not integrate the ψ_m and ψ_s of PEG media to the same degree. Inasmuch as they are not true solutions and the relationship between ψ_w , method of determination, PEG molecular size, and concentration is not consistent, it is difficult to conclude which method is providing an accurate value for ψ_w . A partial explanation for the discrepancy between the two methods may be attributed to the difference in sample temperature at time of measurement. There is no reason to believe that the more linear function obtained using vapor-pressure deficit methods is more accurate than the one obtained using freezing-point depression. Higher concentrations and/or higher mol wt of PEG (longer polymer chains) would reduce the free energy of H_2O proportionally more by matric forces than by osmotic forces. These conditions suggest that the relationship between ψ_w and PEG concentration would be curvilinear. However, large differences in ψ_w estimates exist between the two methods at certain PEG concentrations.

The study presented here does not establish an appropriate method for determining the ψ_w of PEG media. It does point out that, although considerable research in the field of plant- H_2O relations has depended on the use of PEG for simulating water stress, little is known about the behavior of PEG in reducing ψ_w . Furthermore, the possible existence of cation-active polyoxonium ions may affect the availability of nutrients to plants growing in PEH-nutrient media. Future research is needed to determine if long-chain organic molecules have similar effects on ψ_w within living cells.

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